IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Applicant: Karel Newman, *et al.*

Serial No.: 08/070,099

Filed: 05/28/93

For: IMMUNOASSAYS FOR DETERMINING VITAMIN B12 AND REAGENTS AND KITS THEREFOR

Art Unit 1806

Atty. Docket No. 19867.1.0

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Hon. Commissioner of
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Attention: Board of Patent Appeals and Interferences

AMENDED APPELLANT'S BRIEF (37 CFR 1.192)

An Appellant's Brief was filed May 15, 1995 in furtherance of the Notice of Appeal filed in this case on March 15, 1995. In a communication mailed June 20, 1995, the Examiner indicated that a new Brief should be filed to comply with recent amendments to the provisions of 37 CFR 1.1.92(c). Accordingly, this Amended Brief is filed with the period set by the communication to end July 20, 1995.

The fees required under § 1.17(f) and any required petition for extension of time for filing this brief and fees therefor are dealt with in the previously filed Transmittal of Appeal Brief. In the event any further fees are in order in connection with this Amended Brief, authorization is hereby provided to debit Deposit Account No. 06-1910.

This brief contains these items under the following headings and in the order set forth below (37 CFR 1.192(c)):

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X. SUMMARY

I. REAL PARTY IN INTEREST

The real party in interest in this application is Diagnostics Pasteur, since renamed Pasteur Sanofi Diagnostics, as assignee of parent application Serial No. 07/07/682,060, filed April 9, 1991.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences known to appellant, appellant's legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS (37 CFR 1.192(c)(1))

The status of the claims in this application are:

A. TOTAL NUMBER OF CLAIMS IN APPLICATION

Claims 1-10 are pending in the application.

B. STATUS OF ALL THE CLAIMS

Claims 1-10 have been finally rejected in the Office Action mailed November 15, 1994.

C. CLAIMS ON APPEAL

Claims 1-10 are on appeal.

IV. STATUS OF AMENDMENTS (37 CFR 1.192(c)(2))

An Amendment was filed August 9, 1994 in this application, concurrent with a telephonic interview between Applicant Jane Schmidt, the Examiner, and the attorney of record. Thereafter, a final Office Action, enclosing an "Examiner Interview Summary Record", was mailed November 15, 1994. The final Office Action serves as the basis for the present appeal.

The claims on appeal have not been amended from those originally filed May 28, 1994 in the form of the above-captioned CIP application, although in the Office Action mailed April 4, 1994, claims 9-11 were renumbered as claims 8-10 to make numbering consecutive in accordance with Rule 1.126.

V. SUMMARY OF INVENTION (37 CFR 1.192(c)(3))

The present invention relates to an immunoassay for determining the level of vitamin B12 in a sample. Vitamin B12 is a water-soluble vitamin critical to such functions as amino acid metabolism and red blood cell production by the body. The accurate and rapid assessment of the level of B12 in a biological sample can provide important physiological information.

Since B12 is an unusually large and highly-charged vitamin, the body has developed a unique system for the intestinal uptake and transport of the vitamin. Generally, B12 is taken up by the body in a form bound to a glycoprotein present in the stomach and known as intrinsic factor ("IF"). Once B12 has been effectively transported into the epithelial cells of the stomach, the IF remains behind in the lumen.

Assays for B12 often make use of the unique binding relationship between B12 and IF. In particular, antibodies specific for the B12 binding site of IF have been previously prepared. As such, the antibodies are capable of mimicking the binding of B12 and thereby "competing" with B12 for binding to IF. In such a "competitive binding" assay, one can determine the extent to which antibody binding is affected by the presence of any B12 that may be present in a sample. That data, in turn, can be used to determine the concentration of B12 itself.

Phrased another way, traditional antibodies are generally "directly competitive" with B12, in that the binding site on IF will not be able to bind vitamin if previously bound with antibody, and vice versa. Once bound to either B12 or antibody, the IF binding site only becomes available again if the first binding partner spontaneously disassociates itself from binding with IF. In that case, and as with any unbound IF, antibody and vitamin would compete with each other for binding to the unbound IF, in a manner dependant on their relative concentrations and binding affinities for IF.

The present invention provides a unique and unexpected type of antibody for performing a B12 assay. Applicants have coined the term "allosteric competitive" to describe the function displayed by the present antibody. As one element of that function, and not unlike traditional "directly competitive" antibodies, the allosteric antibody of the invention demonstrates the ability to bind to IF only in the absence of B12.

In clear contrast to traditional antibodies, however, Applicants have found that B12 can nevertheless bind to IF even if that IF has already been bound to the allosteric antibody. In so doing, the B12 is able to cause the release of previously bound antibody. As described

below, however, this does not necessarily mean that the B12 and allosteric antibody are directly competitive or that they bind at or near the same site.

In other words, the addition of B12 is able to "bump" previously bound allosteric antibody from its bound position with IF. Again in contrast, a previously bound "directly" competitive antibody would be largely unaffected by the addition of B12. A traditional IF-antibody complex would need to first dissociate at its spontaneous dissociation rate before B12 and antibody could directly compete for binding with the free site.

Although the mechanism for such an allosteric relationship is not fully understood, the fact that this relationship exists is nevertheless clear and supported by the data. When understood and considered fairly, the data provided by Applicants supports the conclusions set forth above. The unique features provided by an allosteric antibody of the present invention are unexpected and provide a number of advantages and options unavailable with traditional "directly competitive" antibodies.

VI. ISSUES (37 CFR 1.192(c)(4))

A. Whether claims 1-10 are unpatentable under 35 U.S.C. 112, first paragraph as failing to adequately teach how to make and/or use the invention, i.e,. for failing to provide an enabling disclosure and failing to adequately describe the invention.

B. Whether claim 1 is unpatentable under 35 U.S.C. 102(b) as being anticipated by Smolka *et al.*

C. Whether claims 2-8 are unpatentable under 35 U.S.C. 103 over Galfre *et al.* in view of Chen *et al.*

D. Whether claims 9-10 are unpatentable under 35 U.S.C. 103 over Ellis *et al.*

VII. GROUPING OF CLAIMS (37 CFR 1.192(c)(5)

For each ground of rejection which applies to more than one claim, the rejected claims will be considered to stand or fall together in order to facilitate the issues considered in the present appeal.

VIII. ARGUMENTS

A. ARGUMENT: OBJECTION AND REJECTION UNDER 35 U.S.C. 112, FIRST PARAGRAPH

The objection and rejection under Section 112 provides the key area of disagreement between Applicants and the Examiner. The rejection revolves around an understanding of the experimental protocol employed by Applicants, and in turn, the use of the data generated to support the patentability of the present invention.

Understanding the data presented by Applicants requires that there be a clear understanding of the experimental methodology used. In the event the Board agrees with Applicants' explanation of the methodology and interpretation of the data, it will be apparent that the basis underlying each rejection will be readily traversed.

Applicants propose that the data describes an antibody that performs in the "allosteric competitive" manner described above. The Examiner concludes instead that the antibodies described and exemplified by Applicants are of the traditional "directly competitive" type.

Figure 2 of the present specification provides ample support for the conclusions asserted by Applicants. The data presented in Figure 2 are the result of the BIAcore method described in the specification. A BIAcore experiment measures the optical effects of proteins bound to the surface of a biosensor chip within a flow cell. Only those proteins (or complexes thereof) that are of sufficient size and actually bound to the chip will be detectable.

Vitamin B12, having a formula weight of 1355 is itself too small to affect the signal generated in the BIAcore experiment. Since the signal is proportional to the mass of bound protein, B12 alone does not affect that signal, leaving only allosteric antibody and IF as being large enough to be detected, if bound to the chip.

In operation, the BIAcore technique described in the specification can be summarized as follows:

1) A BIAcore flow cell is first established having the allosteric (anti-IF) antibody bound to polyclonal (anti-IgG) antibody which, in turn, is covalently immobilized within the cell.

2) Intrinsic factor (IF) is then introduced into the cell, where it is able to bind to the immobilized allosteric antibody in order to form bound IF/allosteric antibody complexes. Any unbound IF is then washed away by the flow within the cell. A baseline signal correlates with the total amount of IF/antibody initially bound.

3) B12 is then introduced into the cell. The data in Figure 2 demonstrates the results achieved with the introduction of increasing concentrations of B12, corresponding

from the top pair of curves (0 micromolar B12), to the bottom pair of curves (50 micromolar B12).

4) It can be seen that with each increase in B12 concentration, the corresponding "relative response" values decrease. A decrease in the detectable signal can only mean that the previously bound IF is becoming disassociated from the immobilized allosteric antibody, by virtue of the presence of B12.

It is important to remember that each of the above steps are performed with the continual delivery of respective reagent solutions. As a result, no unbound component (i.e., B12 or IF) remains in the flow cell, and any component that becomes disassociated from its immobilized form will also be washed from the cell.

In view of the continual flow of buffer through the cell, and the binding relationships established, only immobilized complexes (i.e., allosteric antibody/IF or allosteric antibody/IF/B12) will be detectable in the present assay. Any component that is not present in immobilized form such as unbound B12 or IF, or non-immobilized IF/B12 complexes, will be washed from the cell and not detected.

These results show that the complex of allosteric antibody/IF disassociates at an increasing rate with increasing B12 concentration, as compared to the control rate established at 0 micromolar B12. The antibody of the present invention is therefore "allosteric", as defined by Applicants, since it is capable of specifically binding to IF only in the absence of B12, and is released from binding in the presence, and upon the binding, of B12 to IF.

In view of this experimental design, the Examiner's conclusions are unsupported by the data. At page 2 of paper 9, for instance, the Examiner concludes that "the results of

figure 2 would seem to buttress the conclusion that the 585.3A3A8 antibody merely binds at the binding site to B12-intrinsic factor or in a manner that sterically hinders the binding".

If the antibody were to "bind at the binding site" of B12, then B12 would be competitive in the conventional sense with the antibody. If that were true, however, one would not expect to see a change in the rate of IF disassociation from immobilized antibody. Rather, the common binding site on IF would initially be inaccessible to B12, since it would be in a position bound to the immobilized antibody. The IF site would only become accessible at the rate of spontaneous disassociation of IF from antibody. Moreover, any resulting IF/B12 complex would then be washed from the cell undetected, and with no effect on the overall spontaneous disassociation of remaining immobilized complexes. This would be true regardless of the relative affinities of B12 and antibody for binding with IF.

Neither does the data support the Examiner's suggestion that the allosteric antibody merely binds to IF "in a manner that sterically hinders the binding" of the B12. This suggestion would not explain how increasing concentrations of B12 can be effective at causing an increased rate of disassociation of IF previously bound to allosteric antibody.

Applicants propose that the Examiner's conclusions are based on a misunderstanding of certain concepts underlying the test methodology employed. Such misunderstanding is evident, for instance, by the Examiner's assertion that "[i]f the affinity of B12 for IF is higher than antibody for IF, then B12 will indeed "bump" the antibody off and the IF will binding will be dependent on the concentration of B12. (Paper 9, page 3, lines 16-19).

There is no support in the literature, or in logic, for the assertion that a second binding partner can "bump" a first competitive binding partner from its position in a

complex, solely by virtue of the greater affinity of the second binding partner. Only upon the disassociation of the first binding partner, as might occur spontaneously, can the second binding partner use that greater affinity to its advantage.

A brief explanation of the unique nature of the IF/B12 relationship might help the Board to understand the mechanism Applicants believe may underlay the present invention. It appears that IF exists in at least two forms in the body, each having unique biophysical properties. When B12 is bound to IF, the IF protein appears to form a dimer, which is then capable of binding to a specific extracellular receptor. In the absence of B12, IF appears to exist as a monomer that is unable to bind with the receptor.

Applicants suspect that the allosteric antibody of the invention binds to a site that is only exposed in the monomeric form (i.e., when not bound in the form of a dimer to B12). Since the antibody does not bind to the B12 binding site *per se*, but only binds to IF in the absence of B12, it follows that the antibody binds to a distinct "allosteric" site.

Applicants chose the term "allosteric" in view of similar properties that have been observed in the context of enzyme activity. The activity of many enzymes can be controlled by positive or negative effectors that bind at sites distinct from an enzyme's own active site. Because these effectors bind at remote sites, such an enzyme is generally able to simultaneously bind its substrate at the active site as well. Binding of the substrate, however, often results in a change of the affinity of the enzyme for its substrate.

Although not being bound by this theory, the data and functional interpretation of the allosteric antibody support this conclusion. The Examiner's disbelief, in order to be upheld,

must be based on facts and arguments having more substance than his mere unwillingness to understand or agree with the Applicants' interpretation of the data.

The Examiner's assertion regarding the need for undue experimentation is a further result of this unwillingness. It is clear that the description is amply sufficient to allow those skilled in the art to prepare, identify and distinguish allosteric antibodies in the manner presently claimed. The specification fully describes a particular extraction and screening method that makes use of and accommodates the unique binding parameters of the IF-B12-antibody relationship. Once antibodies that bind IF only in the absence of B12 have been identified, those skilled in the art will appreciate the manner in which such methods as the BIAcore method can be used to identify those that are allosteric competitive antibodies.

B. ARGUMENT: REJECTION UNDER 35 U.S.C. 102(b)IN VIEW OF SMOLKA *ET AL.*

As with each of the art-based rejections, the Examiner's reliance on Smolka *et al.* is largely based on the Examiner's unwillingness to accept the principle of "allosteric competitive" antibodies. A simple reading of the rejection makes clear that it is little more that a paraphrasing of the issues and assertions raised under Section 112. Resolution of those issues should serve to effectively traverse the rejection based on Smolka *et al.*

Smolka *et al.* merely describe how to obtain monoclonal antibodies to IF. They do not describe the selection or production of allosteric competitive antibodies as presently claimed. Similarly, Smolka *et al.* do not describe extracting B12 from culture supernatant

before selecting hybridomas that secrete antibodies to IF. As a result, any allosteric competitive antibodies that may be present would not be selected or identified as such.

The Examiner's "invitation" to show by side-by-side comparison that "the prior art antibody would not inherently contain the allosteric characteristics claimed" is unrealistic and unfounded in the law. Applicants cannot be expected to prove a negative, i.e., to obtain and compare every prior art antibody described as being directly competitive in order to show that they do not function in the manner presently claimed.

C. ARGUMENT: REJECTION UNDER 35 U.S.C. 103 OVER GALFRE *ET AL.* IN VIEW OF CHEN *ET AL.*

With respect to this rejection of claims 2-8, Galfre *et al.* merely describe general methods for the preparation of antibodies, while Chen *et al.* describe a radioassay that involves the use of "pure IF". Neither reference, either alone or in combination, teach or suggest the preparation or use of allosteric competitive antibodies in the manner presently claimed.

If anything, references such as Galfre *et al.* and Chen *et al.*, and for that matter Smolka *et al.*, support the patentability of this invention by undermining the assertions made by the Examiner under Section 112. References such as these demonstrate the availability of skills, processes, and reagents available to those skilled in the art, together with the present teaching, for use in making and using antibodies as presently claimed.

D. ARGUMENT: REJECTION UNDER 35 U.S.C. 103 OVER ELLIS *ET AL.*

The Examiner's reliance on Ellis *et al.* is respectfully traversed. Although the initial statement of the rejection does not appear to be consistent with the text of the rejection (i.e., regarding reliance on the Galfre *et al.* and Chen *et al.* references), these references are nevertheless distinguished for the reasons provided above and previously. Ellis *et al.* adds nothing to remedy the fundamental defects of Galfre *et al.* and Chen *et al.* described above.

The Examiner suggests that the allosteric competitive antibodies of the invention can be used in place of the receptor of Ellis *et al.* There is certainly no suggestion in Ellis *et al.* of such a substitution, nor is it clear what purpose such a substitution would achieve.

Regardless of what may or may not be fairly suggested by Ellis *et al.*, it is quite clear that the reference fails entirely to teach or suggest the use of an allosteric antibody as described and claimed by Applicants.

IX. APPENDIX OF CLAIMS INVOLVED IN THE APPEAL

The text of the claims on appeal are:

1. A composition useful for determining the presence of vitamin B12 in a sample, the composition comprising an antibody that is capable of specifically binding to intrinsic factor only in the absence of vitamin B12, and that is released from binding in the presence, and upon the binding, of vitamin B12 to intrinsic factor.

2. A kit for determining the presence of vitamin B12 in a sample, the kit comprising intrinsic factor and labelled antibody, the antibody being one which will specifically bind to the intrinsic factor in an amount related to the amount of vitamin B12

present in the sample, wherein the antibody is capable of binding to intrinsic factor only in the absence of vitamin B12, and is released from binding in the presence, and upon the binding, of vitamin B12 to intrinsic factor.

3. The kit of claim 2 further comprising a second antibody which will specifically bind to intrinsic factor without regard to the presence or absence of vitamin B12.

4. The kit of claim 3 wherein the second antibody is bound to a solid phase support.

5. A kit for assaying for vitamin B12 in a sample comprising (a) solid phase support to which is bound a predetermined amount of an antibody that is capable of specifically binding to intrinsic factor only in the absence of vitamin B12, and that is released from binding in the presence, and upon the binding, of vitamin B12 to intrinsic factor, and (b) a predetermined amount of a labelled intrinsic factor.

6. The kit of claim 5 wherein the label is alkaline phosphatase and further comprising a substrate to detect the presence or amount of the label.

7. A method of obtaining a monoclonal antibody capable of binding to intrinsic factor only in the absence of vitamin B12, and being released from binding in the presence, and upon the binding, of vitamin B12 to intrinsic factor, comprising the steps of:

a) immunizing an animal with substantially purified intrinsic factor;

b) isolating splenic lymphocytes from the immunized animal;

c) fusing the isolated splenic lymphocytes with a plasmocytoma cell line to obtain a plurality of hybridoma clones which secrete antibody;

d) extracting free vitamin B12 from a predetermined amount of culture supernatant containing antibody from each hybridoma clone;

e) contacting a first sample of each extracted antibody-containing supernatant with intrinsic factor in the presence of vitamin B12;

f) contacting a second sample of each extracted antibody-containing supernatant with intrinsic factor in the absence of vitamin B12;

g) contacting an enzyme labelled antibody which specifically binds to immunoglobulin with each of the first and second samples;

h) detecting the presence of labelled antibody present in each of the first and second samples; and

i) isolating the hybridomas which secrete antibodies which bound to intrinsic factor only in the absence of vitamin B12.

8. The method of claim 8 wherein the extraction step (d) is performed using dextran coated charcoal.

9. A diagnostic assay method for vitamin B12 in a liquid sample comprising:

(a) contacting the sample with a known amount of labelled intrinsic factor and a known amount of an

antibody bound to a solid phase which specifically binds to intrinsic factor in an amount related to the presence or amount of vitamin B12 in the sample, said antibody being capable of binding to a site on intrinsic factor that is distinct from the site on intrinsic factor to which vitamin B12 binds and which binds to intrinsic factor only in the absence of vitamin B12, and that is released from binding in the presence, wherein the intrinsic factor will specifically bind to vitamin B12 in the sample to form a vitamin B12-intrinsic factor complex;

(b) separating the vitamin B12-intrinsic factor complex; and

(c) determining the amount of vitamin B12 by measuring the amount of label associated with the vitamin B12-intrinsic factor complex or the amount label bound to the antibody on the solid phase.

10. The method of claim 9 wherein the antibody is a monoclonal antibody.

X. SUMMARY

For the foregoing reasons, it is submitted that the Examiner's rejections of claims 1-10 were erroneous, and reversal of his decision is respectfully requested.

Respectfully submitted,

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